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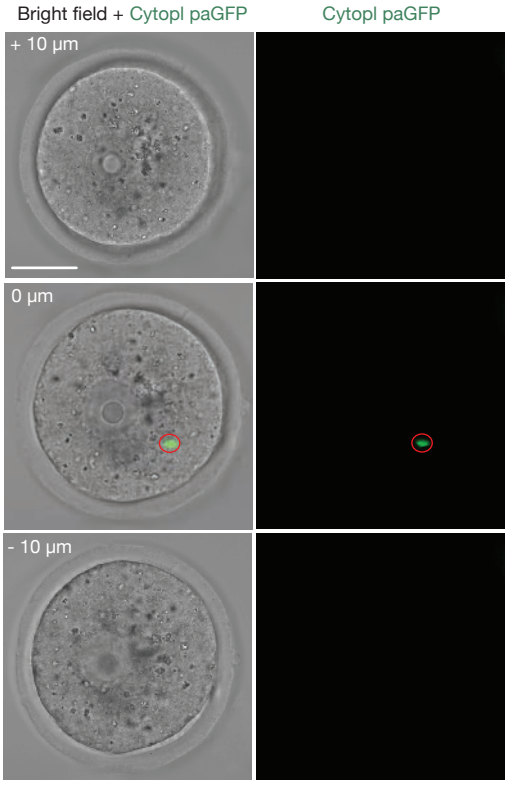

Figure S1 Photoactivation of paGFP in a fixed mouse embryo. A one-cell stage embryo was microinjected with cytoplasmic paGFP RNA and fixed 4 hours after injection in paraformaldehyde. A small ROI (red circle) was photoactivated at a single z-plane. Immediately after photoactivation paGFP fluorescence is confined to the ROI. Fluorescence is not observed at z-planes localized 10 µm above or below the plane of photoactivation (0 µm). Scale bar corresponds to 20 µm.

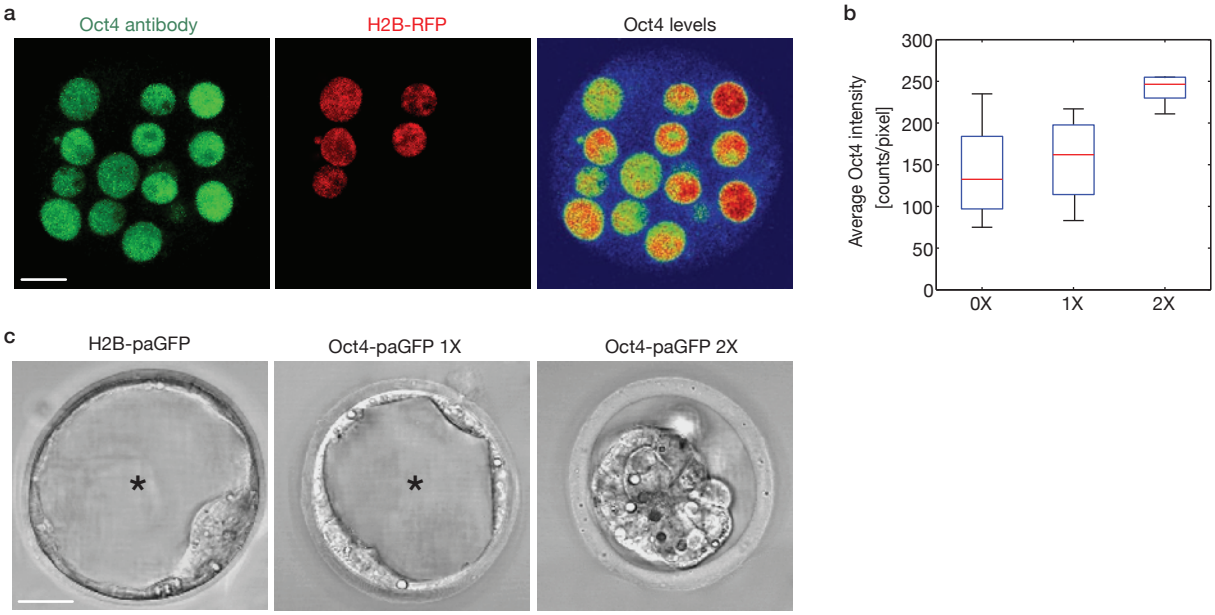

Figure S2 Oct4 expression levels and distribution in mouse embryos. **a**, Oct4 antibody staining shows mostly nuclear localization of Oct4 in z-plane projections encompassing entire cell nuclei. Throughout our study, one cell of the 2-cell stage embryo was microinjected with Oct4-paGFP (2 ng/µl) DNA and H2B-RFP RNA, respectively. All daughter cells derived from the original injected cell are marked by H2B-RFP in their chromatin (red). Note that in the injected H2B-RFP-positive cells total Oct4 expression (green) is similar to that in non-injected H2B-RFP-negative cells. **b**, Boxplot of quantified Oct4 expression levels. At 2 ng/µl (the 1X concentration used in the FDAP experiments) Oct4 is expressed within the physiological range of endogenous Oct4 (0X). At 4 ng/µl (2X) there is a ~1.8-fold increase in Oct4 levels, and the median expression value (red line in graph) is outside the physiological range. **c**, Effects of Oct4-paGFP expression on embryo development. Embryos injected with 2 ng/µl of photoactivatable Oct4-paGFP develop normally to blastocyst stage and form the blastocoel cavity (asterisk). At 4 ng/µl (2X) Oct4-paGFP affects embryonic development and the blastocoel cavity is not formed. A control H2B-paGFP construct injected at 4 ng/µl does not affect embryo development. Scale bar corresponds to 15 µm.

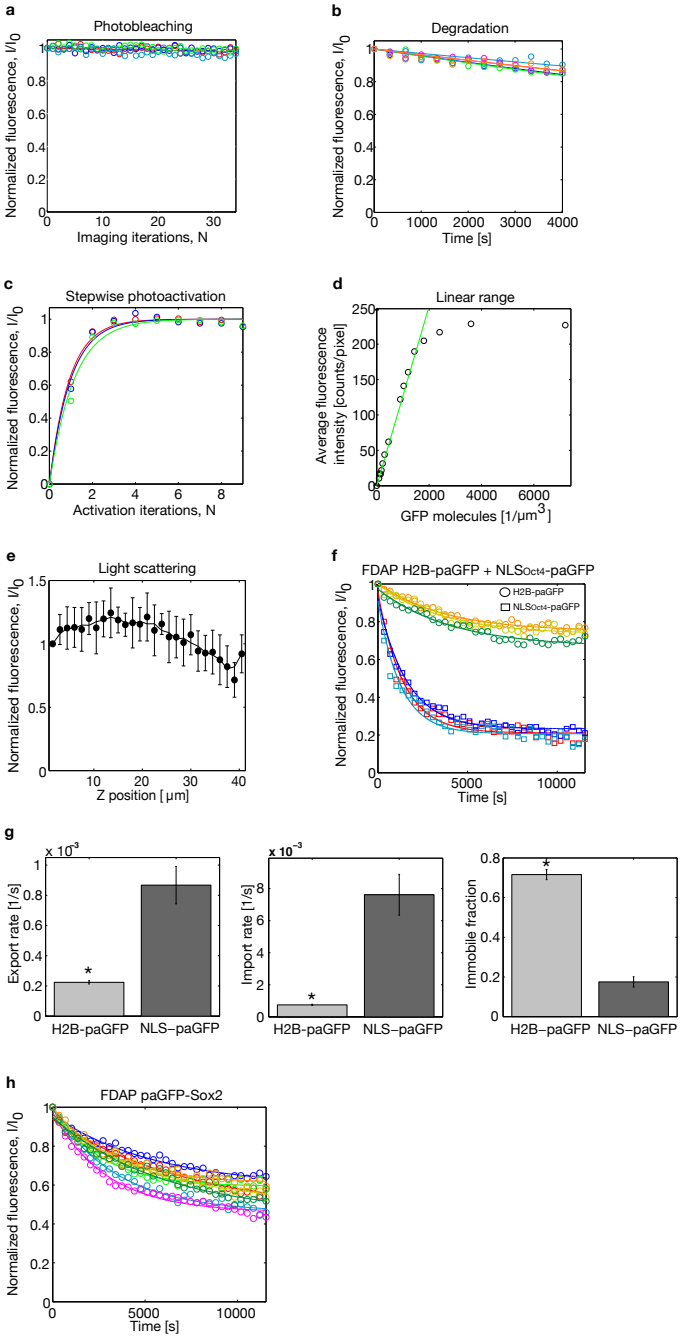

Figure S3 Control experiments for the FDAP assay. **a**, Bleaching in the course of imaging. The contribution of photobleaching by the imaging beam was determined by continuous imaging of Oct4-paGFP fluorescence within a single photoactivated ROI localized to the cell nucleus. **b**, Degradation of photoactivated Oct4-paGFP within the entire cell. Both the cell nucleus and the entire cytoplasm of single cells were photoactivated and the average fluorescence intensity in the whole cell was quantified over time. **c**, Stepwise photoactivation. Photoactivation of Oct4-paGFP in single cell nuclei shows that 2-3 illumination iterations are sufficient to obtain a maximum fluorescence intensity value within the cell nucleus. **d**, Calibration of fluorescence intensity to GFP concentration. GFP in solution at different concentrations was imaged under the same conditions as in the FDAP assay. Three independent measurements for each concentration are shown. A linear regression fit to the first 11 data points is represented by a green line. **e**, Light scattering effect in live mouse embryos. Embryos were microinjected with cytoplasmic paGFP and the average fluorescence intensity was quantified at different z-planes following photoativation of the entire embryo. Light scattering starts affecting fluorescence intensity at ≥ 30 µm distance to the initial focal plane. **f**, FDAP analysis of H2B-paGFP and NLS_{Oct4}-paGFP. H2B-paGFP or NLS_{Oct4}-paGFP were microinjected into 1 cell at the 2-cell stage, photoactivated within single cell nuclei at the 8-cell stage and imaged with the same conditions described for Oct4-paGFP. **g**, Quantification of H2B-paGFP and NLS_{Oct4}-paGFP nuclear export, import and immobile fraction. **h**, FDAP analysis of paGFP-Sox2. paGFP-Sox2 was microinjected into 1 cell at the 2-cell stage, photoactivated within single cell nuclei at the 4- to 8-cell stage and imaged with the same conditions described for Oct4-paGFP. Asterisks show statistically significant differences. Error bars show standard deviations.

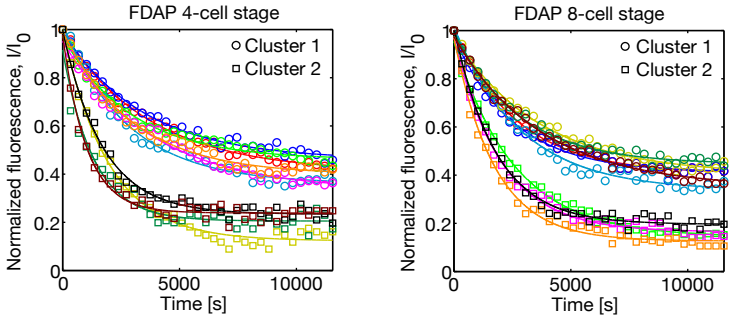

Figure S4 Oct4-paGFP kinetic behaviors at the 4-cell stage and 8-cell stage. FDAP curves for Oct4-paGFP obtained from several cell nuclei of pre-compaction embryos at 4-cell stage (left graph) and at 8-cell stage (right graph). At both developmental stages, cells exhibit two distinct kinetic behaviors, divided into cluster 1 and 2.

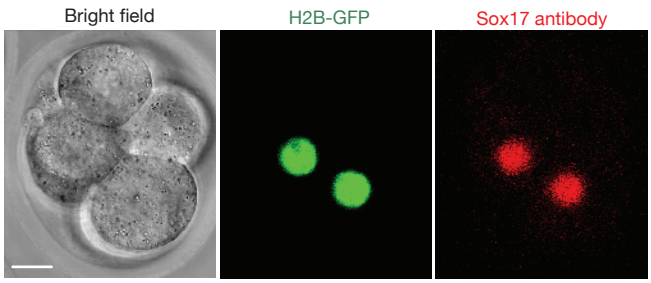

Figure S5 Control experiment for Sox17 expression. In this control experiment, one cell of the 2-cell stage embryo was microinjected with Sox17 (50 ng/µl) RNA and H2B-GFP (10 ng/µl) RNA. All daughter cells derived from the original injected cell are marked by H2B-GFP (green) and exhibit Sox17 protein (red) that localized predominantly in the nuclei. Scale bar corresponds to 15 µm.

Supplementary Movie legends

Movie S1 Selective photoactivation of Oct4-paGFP in a live mouse embryo. Three dimensional view and animated 180° rotation of an 8-cell stage embryo before and after photoactivation of Oct4-paGFP within a single cell nucleus. The appearance of a red circle indicates the ROI photoactivated with 820 nm light. The bright field and the H2B-RFP label show the position of cells in the embryo that express photoactivatable Oct4 before and after photoactivation. Note that photoactivated Oct4 is confined to the cell nucleus immediately after photoactivation.

Movie S2 Imaging Oct4-paGFP in single cells. Time-lapse imaging of two representative photoactivated cell nuclei obtained from a typical FDAP experiment. The cell nuclei belong to two different cells of a pre-compaction, 8-cell stage embryo. The time-lapse sequence was made from a projection of 5 individual z-planes encompassing each cell nucleus. The two nuclei were aligned for comparison. The nucleus on the left panel exhibits the Oct4-paGFP kinetic behavior characteristic of cells in cluster 1, while the nucleus on the right panel displays the behavior of cluster 2 cells.